Short Paper

Antineoplastic Glycoproteins from the Abalone

*Haliotis discus hannai**¹

Hisao Kamiya,*² Koji Muramoto,*² Rina Goto,*²
Nobuhiro Kanno,*² Yoshio Endo,*³
Takuma Sasaki,*³ Nobuo Takasuka,*⁴
and Hiroyuki Uchida*⁴

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Extracts from marine invertebrates have drug-like effects on mammalian and other tissue preparations, including tumors. However, most of these extracts are cytotoxic in action and are toxic to the host. In our previous studies, we found the hot-water extract of the abalone *Haliotis discus hannai* inhibited the growth of allogeneic sarcoma 180 or syngeneic Meth-A fibrosarcoma by both local and systemic injection. The antitumor component(s) showed only weak direct cytotoxicity *in vitro*, suggesting that the active agent(s) stimulate host-mediated response.^{1,2)} The nature of antineoplastic agent(s), however, is not yet clarified. In this report, we describe the characterization of antineoplastic agents from the macromolecular fraction of the abalone.

The purification of antineoplastic compound was carried out as follows. A fraction with a molecular weight greater than 50 kD was prepared from the liquor waste of abalone provided from a manufacturing factory by dialysis and ultrafiltration with a XM-50 membrane. Subsequent column chromatographies on DEAE-Sepharose CL-6B and DEAE-Sephacel according to the previous method²⁾ afforded fraction 9 which showed the highest tumor inhibition ratio. It was further purified on a column of Sephacryl S-300 column equilibrated with 10 mM phosphate buffer containing 0.15 M NaCl (pH 7.4) to yield 4 fractions; 9-1 (yield 34.2%), 9-2 (10.8%), 9-3 (6.6%), and 9-4 (33.6%) according to the range of molecular weights (Fig. 1). The fractions were injected into the lesion at a dose ranging from 0.5 to 1.0 mg/mice on days 5, 7, and 9 after inoculation of sarcoma 180 cells (about 2×10^6 cells) into the right flank of the ICR mice, and were evaluated for antitumor activity at the end of the 5th week, as reported previously.²⁾

Fraction 9-1 and 9-2 showed inhibition ratios of 85.1% and 76.1% at doses of 1.0 mg/mice and 0.5 mg/mice, respectively. These fractions also caused complete disappearance of the tumor in 2 out of 6 mice. On the other hand, lower molecular weight fractions, fractions 9-3 and 9-4, showed low antitumor activity. In fast protein liquid chromatography analysis using a Superose 6 column (Pharmacia), fraction 9-1 gave a broad peak corresponding to about 300 kD, while fraction 9-2 gave one sharp symmetrical protein peak corresponding 140 kD. The purity of the specimens, however, was not ascertained, because they did not give any visible bands on polyacryl amide gel or cellulose acetate strip by staining with various reagents after electrophoresis (data not shown).

Fig. 1. Sephacryl S-300 column chromatography of partially purified abalone antitumor agent (fraction 9).

Sample (280 mg protein) was applied to a column (3.2 × 85 cm) of Sephacryl S-300. The column was eluted with 10 mM phosphate saline (pH 7.4). Fraction size was 4 ml. Fractions were pooled as indicated by horizontal bars. Arrows indicate the approximate positions of marker proteins.

Fraction 9-1 was a glycoprotein which contained 39.6% of total carbohydrate as glucose by the phenol-sulfuric acid method and 34.5% of total protein as egg albumin by the Lowry method,³⁾ while fraction 9-2 showed 39.0% carbohydrate and 28.4% protein.

Elementary analysis of fraction 9-1 showed C, 40.9; H, 6.05; N, 4.90; S, 0.84; and ash, 11.4% while that of fraction 9-2 was C, 40.6; H, 6.42; N, 5.34; S, 1.82; and ash 4.86%. Ion chromatography on a TSK-Gel IC-anion PW column (Toso) revealed that fraction 9-2 contained about 2.2% sulfate. The treatments with pronase E from *Streptomyces griseus* (Sigma) or sulfatase from *Aerobacter aerogenes* (Sigma) at 37°C for 2 h decreased the antineoplastic activity of fractions 9-1 and 9-2. Also, glycosidase mixed from *Turbo cornutus* (Seikagaku Kogyo) and 0.2 M sodium metaperiodate treatments of fraction 9 destroyed the activity (data not shown). These results indicate that both sulfated-carbohydrate and protein moieties are essential for the appearance of antineoplastic activity of abalone glycoproteins.

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*² School of Fisheries Sciences, Kitasato University, Sanriku, Iwate 022-01, Japan (神谷久男, 村本光二, 後藤利奈, 菅野信弘: 北里大学水産学部).

*³ Cancer Research Institute, Kanazawa University, Takaramachi, Kanazawa 920, Japan (遠藤良夫, 佐々木琢磨: 金沢大学がん研究所).

*⁴ Chemotherapy Division, National Cancer Center Research Institute, Tsukiji, Tokyo 104, Japan (高須賀信夫, 内田博之: 国立がんセンター研究所).